March 30, 2011

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Via email to: whiteld@niehs.nih.gov.

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

HEADQUARTERS
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Re: 76 FR **National Toxicology Program (NTP); Office of Liaison, Policy and Review; Meeting of the NTP Board of Scientific Counselors, request for comments.**

We appreciate the opportunity to comment on behalf of PETA and the more than two million members and supporters of the world's largest animal rights organization. These comments pertain only to NTP's Modified One-Generation Reproduction Study Design.

General Comments

We appreciate NTPs stated desire to incorporate the 90-day repeat dose with the reproductive toxicity study, and while this Modified One-Generation Study may use fewer animals than NTP's standard reproductive assessment by continuous breeding. However, ***we object in the strongest terms possible to development of this new study design as it use more animals than are used in all other reproductive toxicity study designs, including the standard two-generation reproductive toxicity study.*** Rather than a "Modified One-Generation Reproductive Study Design," the correct title for this study would be a "Modified Two-Generation Reproductive Study Design."

We are extremely concerned about NTP's increasing focus on animal testing for developmental and reproductive endpoints. While we understand the concern regarding potential human health effects of lifetime exposure to industrial and other chemicals, ***there is no assurance that tests involving longer exposure (i.e. perinatal) or using additional animals will prove any more relevant to human health protection than the existing shorter versions***. At the same time, these extended protocols have serious ramifications on the welfare of the animals involved. If NTP continues down this road, any use of these prolonged exposure tests should be contingent on retrospective analyses of data from the first such tests to assess performance and review the value added of prolonged treatment and additional animals.

Lastly, the description of the NTP Modified One-Generation Study provided is not of sufficient detail to adequately evaluate the impact on animals or offer detailed suggestions for reducing animal use.

Specific Comments

The need for better evaluation of postnatal outcomes – improvements made to our multi-generation study.

The comparison of analysis of 250 fetuses (in prenatal developmental tests) vs. 40 adults in the multi-generational study is made to support the statement that "the ability to evaluate (both detection and analysis of dose response) abnormalities of the reproductive tract...was determined to be underpowered by several research groups." The proposal does not discuss whether, in evaluating more offspring per litter, it is referring to F1 or F2 animals, nor does it discuss what number of animals the agency believes would improve statistical power. Furthermore, the ability to discern differences in a given endpoint is related to the sensitivity of that endpoint; therefore, statistical power is end-point-specific, and such an analysis must be performed relative to specific endpoints. Following such an analysis, animal use should be minimized to obtain the desired statistical power.

Movement to the inclusion of perinatal exposure periods in NTP rat cancer bioassays – need for a suitable preliminary study.

This paragraph seems to indicate that a 90-day repeat dose study to determine starting doses for the multigenerational assay has been modified to include more detailed information on reproduction and development and will also be used to determine starting doses for the rodent cancer bioassay. It is not clear if this is a different, additional assay to the Modified One-Generation Study or is in fact a description of the subchronic toxicity cohort of the proposed NTP study design. It is also stated that this study would be performed *in lieu* of other "stand alone" reproductive studies, but it is not clear what these other studies are since the one-generation study is rarely performed, reproductive/developmental screen is not usually considered "stand alone." Presumably this refers to the standard two-generation study) These two points should be clarified.

Other international efforts to develop new reproductive toxicity study designs.

This paragraph discusses the OECD Extended One-Generation Reproductive Toxicity Study (EOGRTS) that came out of the EPA/ILSI/HESI extended one-generation study design.[1] In the current version of the OECD Test Guideline, the neurotoxicity and immunotoxicity are not triggered; in fact there must be justification (either scientific

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[1] Cooper et al., (2006) Crist Rev Toxicol.;36(1):69-98.

[2] OECD Guideline for the Testing of Chemicals: Extended One-Generation Reproductive Toxicity Study. November 2011. http://www.oecd.org/dataoecd/23/10/46466062.pdf (accessed 30 March 2011).

or policy) for waiving these cohorts.[2] In addition, this paragraph indicates that the impetus for designing a reproductive study that avoids the second generation was solely to reduce animal numbers. While minimizing animal use may have been a consideration, ***a major factor in the redesign was the observation that the second generation provided no added value in terms of information for regulatory use.*** As part of the studies leading to the OECD test guideline, a retrospective analysis of 498 substances (including industrial chemicals as well as pesticides) has verified this finding.[3] This information should be added to this discussion.

The proposed NTP design

This study design involves exposure of pregnant females throughout pregnancy (the P generation, minimum of 20 animals per dose), lifetime exposure of the F1 (300 animals per dose), and generation of two cohorts of F2 animals (developmental and reproductive, 300 animals each per dose). This represents an increase of more than 1200 animals over the standard two-generation reproductive toxicity study, and 2400 animals more than the base EOGRTS study design (**Table 1**).

Advantages of the NTP proposal over the draft OECD design

Justification for the proposed NTP study design includes the criticism that the 10-week pre-mating exposure of P males does not cover all aspects of spermatogenesis. A simpler approach to ameliorate this concern would be to extend the pre-mating parental exposure (also discussed during the OECD EOGRTS study design). A second concern is that the second generation (F2) is not routinely produced; however, as has been extensively discussed and heavily documented, the second generation rarely, if ever, provides additional information that would have been missed in the first generation. In addition, the EOGRTS study design provides the option to generate an F2 where scientifically justified or required by the relevant regulatory framework. Therefore, routine generation of 1200, or in this case 2,400 (not including parental males or extra pregnant females to ensure 20 litters), additional animals is completely objectionable on the basis of animal welfare concerns. A third objection is raised to the use of internal or external triggers to determine whether or not to breed the F1, yet no explanation or rationale is provided to support the need for routine breeding of the F1. Finally, objection is expressed to the use of only 10 animals per sex per dose in the neurotoxicity cohort. If there is scientific and statistical justification for this concern, a simple remedy would be to place additional animals in this cohort.

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[2] OECD Guideline for the Testing of Chemicals: Extended One-Generation Reproductive Toxicity Study. November 2011. http://www.oecd.org/dataoecd/23/10/46466062.pdf (accessed 30 March 2011).

[3] Piersma, A.H. et al. 2011. Combined retrospective analysis of 498 rat multi-generation reproductive toxicity studies: On the impact of parameters related to F1 mating and F2 offspring. Reproductive Toxicology, In Press, Corrected Proof, Available online 3 December 2010.

The use of a unique study design using an unjustifiably large numbers of animals by NTP is not only objectionable from an animal welfare basis, it is a yet another example of NTP's use of non-harmonized study designs which prohibits the use of the resulting information by regulatory and other entities.

Conclusion

The proposed study design involves an extraordinarily large number of animals. Contrary to the claim in the concluding paragraph, this study will use more animals than any study used in any regulatory framework, including the standard two-generation reproductive toxicity study, and represents a shift that runs counter to most other testing programs. The EPA, FDA, and some elements of NIEHS, as well as the OECD and testing approaches for REACH, are all moving toward the minimization and eventual elimination of animal use in chemical assessment yet the NTP is apparently insisting on using the maximum number of animals possible.

Sincerely,

Catherine Willett, PhD
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Table 1: Animals used in NTP study design compared with other reproductive toxicity studies.

Standard Two Generation Study	
Parental males: four groups of 25	100
Parental females: four groups of 25	100
F1 offspring (four groups of 20 litters of 15 pups)	1200
F2 offspring (four groups of 20 litters of 15 pups)	1200
	2600
EOGRTS	
Parental males: four groups of 25	100
Parental females: four groups of 25	100
F1 offspring (four groups of 20 litters of 15 pups)	1200
	1400
NTP Study Design	
Parental females: four groups of 20	80
F1 offspring (four groups of 20 litters of 15 pups)	1200
F2 dev tox offspring (four groups of 20 litters of 15 pups)	1200
F2 repro tox offspring (four groups of 20 litters of 15 pups)	1200
	3680